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Hypothermia and Chloropent Anesthesia Differentially Affect the Flash Evoked Potentials of Hooded Rats

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Robert Dyer and William Boyes

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16. ABSTRACT

Anesthetics and body temperature alterations are both known to alter parameters of sensory-evoked responses. However few studies have quantitatively assessed the contributions of hypothermia to anesthetic-induced changes. Two experiments were performed. In the first, chronically implanted rats were injected with either 0, 0.05, 0.10 or 0.20 ml Chloropent/100 gb.w, while body temperature was maintained. Flash evoked potentials recorded 30 min later showed increased latencies but only minor (not statistically significant) changes in amplitude. In the second experiment the same rats were anesthetized with 0.35 ml Chloropent/100 g b.w. and rectal temperature was systematically varied between 31 C and 37 C. Over the ranges of temperature and anesthetic employed, latencies increased more extensively with hypothermia than with anesthesia. P1N1 amplitude doubled when temperature was lowered to 31 C, but P2N2 and N2P3 amplitudes declined over the same temperature range. Anesthetic-induced changes in peak-to-peak amplitude did not reach statistical significance when body temperature was constant. The findings suggest that previously reported alterations in evoked potentials following anesthesia may have been confounded with hypothermia.

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Hypothermia and Chloropent Anesthesia Differentially Affect the Flash Evoked Potentials of Hooded Rats[1]

ROBERT S. DYER AND WILLIAM K. BOYES

Neurophysiology Branch, Neurotoxicology Division MD-74B
U. S. Environmental Protection Agency, Research Triangle Park, NC 27711

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DYER, R. S. AND W. K. BOYES. *Hypothermia and chloropent anesthesia differentially affect the flash evoked potentials of hooded rats.* BRAIN RES BULL 10(6) 825–831, 1983.—Anesthetics and body temperature alterations are both known to alter parameters of sensory-evoked responses. However few studies have quantitatively assessed the contributions of hypothermia to anesthetic-induced changes. Two experiments were performed. In the first, chronically implanted rats were injected with either 0, 0.05, 0.10 or 0.20 ml Chloropent/100 g b.w., while body temperature was maintained. Flash evoked potentials recorded 30 min later showed increased latencies but only minor (not statistically significant) changes in amplitude. In the second experiment the same rats were anesthetized with 0.35 ml Chloropent/100 g b.w. and rectal temperature was systematically varied between 31°C and 37°C. Over the ranges of temperature and anesthetic employed, latencies increased more extensively with hypothermia than with anesthesia. P1N1 amplitude doubled when temperature was lowered to 31C, but P2N2 and N2P3 amplitudes declined over the same temperature range. Anesthetic-induced changes in peak-to-peak amplitude did not reach statistical significance when body temperature was constant. The findings suggest that previously reported alterations in evoked potentials following anesthesia may have been confounded with hypothermia.

Hypothermia Anesthesia VER VEP Evoked potentials Chloropent FEP

EVOKED potentials have been used to characterize the consequences of exposure to drugs and toxic substances [5, 8, 19]. When properly recorded and interpreted, alterations in evoked potentials may be used to infer alterations in basic physiological mechanisms [10]. Often, however, the contribution of anesthetics and/or hypothermia to drug- or toxicant-induced alterations in evoked potentials is ignored.

In view of the frequency with which electrophysiological studies of neurotoxicity use anesthetics, we considered it appropriate to quantify the influence of the common rat anesthetic, Chloropent, on one frequently used physiological measure of neurotoxicity, the visual evoked response (VER). The influence of rectal temperature upon the VER was examined for two reasons. First, some studies which have used anesthetized preparations to assess neurotoxicity have not provided rigid control of body temperature. Because anesthetic treatment typically lowers body temperature, and because hypothermia is known to affect peripheral conduction velocity [3,6], central conduction velocity [22] and evoked potentials [2], the effects of anesthetics or neurotoxicants may have been confounded with hypothermia (e.g., [5, 13, 14, 19]). This study was designed to determine the consequences of allowing body temperature to fluctuate. Second, some neurotoxic substances may produce alterations in rectal temperature, even in awake animals [9]. The present study was therefore done to illustrate the consequences of failure to consider changes in rectal temperature. While it would be ideal to manipulate rectal temperature independent of anesthesia, the procedure for doing so would have been technically difficult and would have added considerable stress to the animal. It was therefore determined that despite the limitations imposed upon data interpretations, anesthesia would provide the most acceptable means by which rectal temperature could be manipulated.

In addition, the present study documents the normal time course of hypothermia produced by an anesthetic dosage of Chloropent in rats at an ambient temperature of 22°C.

METHOD

This study contains two experiments: an electrophysiological experiment, during which evoked potentials were recorded at various anesthetic levels and rectal temperatures, and a rectal temperature experiment, during which rectal temperature was monitored following anesthesia. The electrophysiological experiment will be described first.

[1]This manuscript has been reviewed by the Health Effects Research Laboratory, U. S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not indicate endorsement or recommendation for use.

Electrophysiological Experiment

Fourteen adult Long-Evans hooded rats (n=8 male; n=6 female) were obtained from Charles River Breeding Co. (Portage, WI). The rats were individually housed with free access to food and water throughout the experiment. At least two weeks before testing, the recording electrodes (00–90×1/16" stainless steel screws) were implanted under Chloropent anesthesia (0.75 ml/100 g b.w., IP). The ground and reference electrodes were screws placed 5 mm anterior and 2 mm left and right of bregma. The visual cortex electrode was placed 6 mm posterior to bregma and 3 mm lateral to the midline. All electrode leads were connected to an Amphenol receptacle which was cemented to the skull using dental acrylic. Following the procedure, each animal was administered 100,000 units penicillin G and returned to its home cage.

Recording Procedure

To ensure constant pupillary dilations, 1% atropine was instilled in the eyes 30 minutes prior to all recording sessions. Recordings made during the anesthetic portion of the experiment (see below) were obtained while animals were placed in a box with mirrors on 3 walls and a Grass strobe on the 4th (see Dyer *et al.* [10] for details). At the time of recording, the receptacle on the skull was connected to the amplifiers via Microdot mininoise cable. High and low filter settings were 1 kHz and 0.8 Hz, respectively. Amplified waveforms were averaged (n=128) by a PDP 11/70 computer (2500 Hz sample rate, 0.4096 sec epoch). The averaged waveforms were displayed on a Tektronix 4054 terminal, where peak latencies and peak-to-peak amplitudes were measured to the nearest 0.1 msec and 0.1 uvolt, respectively. Hard copies of the waveforms were obtained on a Tektronix 4631 hard copy unit. All averaged waveforms were also stored on disk. Stimuli were presented by the Grass strobe at 0.5 Hz and an intensity of 16 (peak power 4.53×10^7 lux relative to background illumination). Because of logistics involved in controlling and measuring rectal temperature, it was not possible to use the same recording chamber for both phases of the electrophysiological experiment. The recording environment used during the hypothermia phase was designed to be as similar to the mirrored chamber as possible. To allow orientation of the strobe lamp in a manner similar to the mirrored chamber, the rat's head was held up with a stereotaxic unit, and the Grass lamp was centered 4.0 cm away from the eye contralateral to the recording electrode. For both phases of the experiment, ambient temperature was maintained at 30°C, the approximate isothermic temperature for the rat. This allowed reasonable assurance that rats treated with anesthetic would not become hypothermic until additional measures were applied (see below).

Protocol

The electrophysiological experiment was performed in two phases. In the first phase (anesthetic phase) each animal was tested 4 times. The tests consisted of recording the averaged evoked potential 30 min following injection with either 0.0 (saline control), 0.05, 0.10 or 0.20 ml Chloropent/100 g b.w. To maintain a constant injection volume of 0.35 ml fluid/100 g, saline was added to each of the Chloropent concentrations. Chloropent (Fort Dodge) is an anesthetic mixture and dosage is therefore calculated by volume of the mixture instead of weight of the compound. Each ml of Chloropent contains 42.5 mg chloral hydrate and 8.86 mg pentobarbital. Animals were tested twice/week on Tuesdays and Fridays, and the treatment order was randomized across the four test days. Six animals receiving 0.20 ml/100 g were not ambulatory (i.e., did not move about spontaneously). The rectal temperature of these animals was monitored after recording sessions, and found to be normal (~37°C).

In the second (temperature) phase, each of 12 rats (6 male, 6 females) was anesthetized once (0.35 ml chloropent/100 g b.w.) and four averaged evoked potentials were obtained while body temperature was systematically lowered from (1) 37°C to (2) 35°C to (3) 33°C and to (4) 31°C. Immediately following the session at 31°C the animal was warmed and a series of recordings were made at the same temperatures during the ascending temperature period. Thus, each animal had 8 averages recorded, 2 at each temperature. Time between the onset of successive averages was less than 10 min. Data analyses were performed upon the averaged values of the two evoked potentials obtained at each temperature. Cooling of the animals was accomplished by placing an ice-filled rubber glove on their backs, and a shallow dish of cool water placed between a whisperfan and the front of their heads. The average time to complete a single animal's session in phase 2 was about 71.7±4 (SEM) min.

Data Analysis

All analyses were performed using the General Linear Models procedures provided under SAS [12]. For purposes of data analysis the experiments were divided into four datasets: phase 1 (anesthetic) amplitude analyses; phase 1 latency analyses; phase 2 (temperature) amplitude analyses; and phase 2 latency analyses. The amplitude analyses contained the following variables: P1N1 amplitude, N1P2 amplitude, P2N2 amplitude, N2P3 amplitude, and P3N3 amplitude. The latency analyses contained the latencies of peaks P1, N1, P2, N2, P3 and N3 as variables. A repeated measures multivariate analysis of variance (MANOVA, Hotelling-Lawley procedure) was performed upon each of the four datasets (called from BMDP within SAS). To maintain an experiment-wise error rate of 0.05, any one of the four MANOVAs was required to achieve a probability of 0.05/4=0.0125 or less to be considered significant. Since evoked potential parameters may vary as a function of gender [11], each MANOVA tested for effects of treatment (either dose or temperature) and treatment × gender interactions. Significant MANOVAs were followed by repeated measures ANOVAs. Since there were 5 amplitude ANOVAs to be performed, a Bonferroni correction [7] was applied which required a probability of 0.05/5=0.01 or less to be considered significant. Similarly, the latency ANOVAs required a probability value of 0.05/6=0.0083 or less to be considered significant. Significant ANOVAs were followed by Duncan's Multiple Range tests to determine the dosages or temperatures at which significant effects were obtained.

Although the data obtained from the 0.35 ml/100 gm dosage 37°C temperature runs were only included in the temperature (phase 2) analyses, the data are presented on the graphs of both the temperature and anesthetic effects for illustrative purposes.

Chloropent-Induced Hypothermia Experiment

In the second experiment, 14 adult male Long-Evans rats were obtained, housed and maintained as described above.

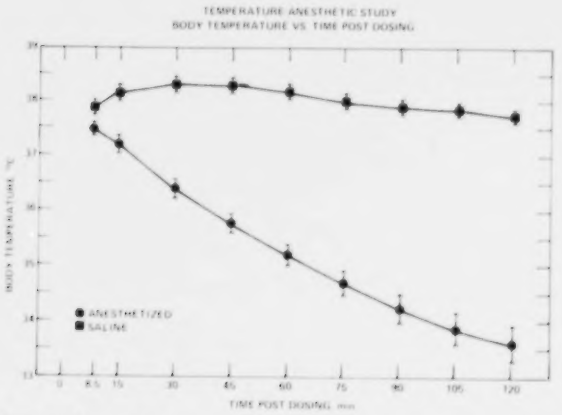

FIG. 1. Averaged rectal temperature for 14 rats at different times following injection of either saline or an anesthetizing quantity of Chloropent (0.35 ml/100 g). Ambient temperature was maintained at 22.04 (±0.02) °C.

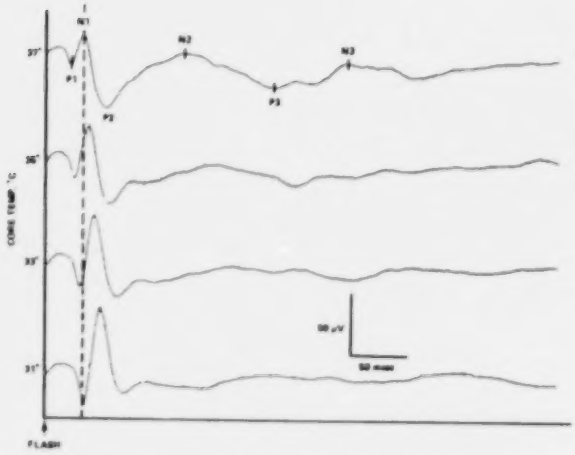

FIG. 3. Averaged VER waveforms from all animals recorded at different rectal temperatures. All animals were anesthetized with 0.35 ml/100 g Chloropent.

The experiment was performed on two separate days. On the first day, the rats were randomly divided into two groups. One group received an anesthetizing injection of Chloropent (0.35 ml/100 g), and the other received an injection of saline (0.35 ml/100 g). All rats were immediately placed in restraining tubes, and rectal thermistor probes were inserted 10.0 cm. Temperature readings were taken immediately, and the time from injection to the temperature reading was recorded. Additional temperature readings were taken at 15 min intervals, beginning 15 min after injection, until 2 hr had elapsed. At this time animals were removed from the restrainers and returned to their home cages. One day later, all animals receiving saline on the first day were anesthetized, and all animals receiving anesthesia on the first day received saline. On both days of the experiment ambient temperature was maintained at 22.04C ±0.02 (SEM). Results were analyzed using a repeated measures ANOVA (2 Variables repeated: dose and time).

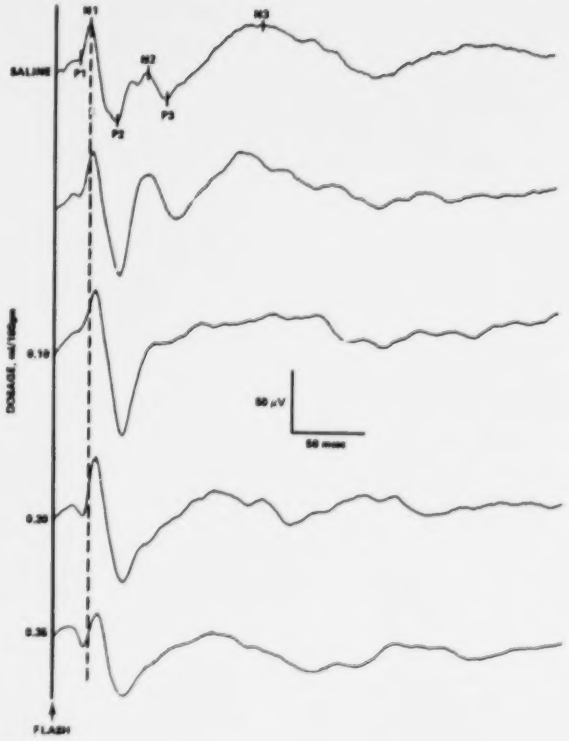

FIG. 2. Averaged VER waveforms from all animals recorded at different levels of Chloropent anesthetic, with body temperature maintained at 37°C.

RESULTS

Chloropent anesthesia produced hypothermia in rats maintained in a 22°C room. Figure 1 illustrates the extent and time course of the changes. Within 2 hr of injection, the average rectal temperature of anesthetized rats dropped to 33.6°C.

Alterations in the flash-evoked potential were produced by the systematic variation of both temperature and dosage of anesthetic. Increasing levels of anesthetic, and decreasing rectal temperature both produced increased latencies of all peaks. Increasing levels of anesthetic produced only modest changes in peak-to-peak amplitudes, but hypothermia produced marked changes in amplitudes. Figure 2 illustrates the effect of variations in level of anesthesia upon the flash evoked responses averaged across all animals in the study. Figure 3 illustrates the effect of progressive hypothermia in anesthetized animals upon flash evoked responses averaged across animals.

Effects of Anesthetic Upon Latencies

The effects of changing level of anesthetic upon latencies of the flash evoked responses are indicated in Fig. 4. The pattern of effects is qualitatively different for early peaks (P1, N1 and P2) than it is for late peaks (N2, P3, N3). Latency to the early peaks is increased (8–16%) at the lowest dosage of Chloropent (0.05 ml/100 g), but further increases in dosage produced little if any further increase in latency. The late peaks also appeared to be affected at the lowest dosage of Chloropent (although only significantly so

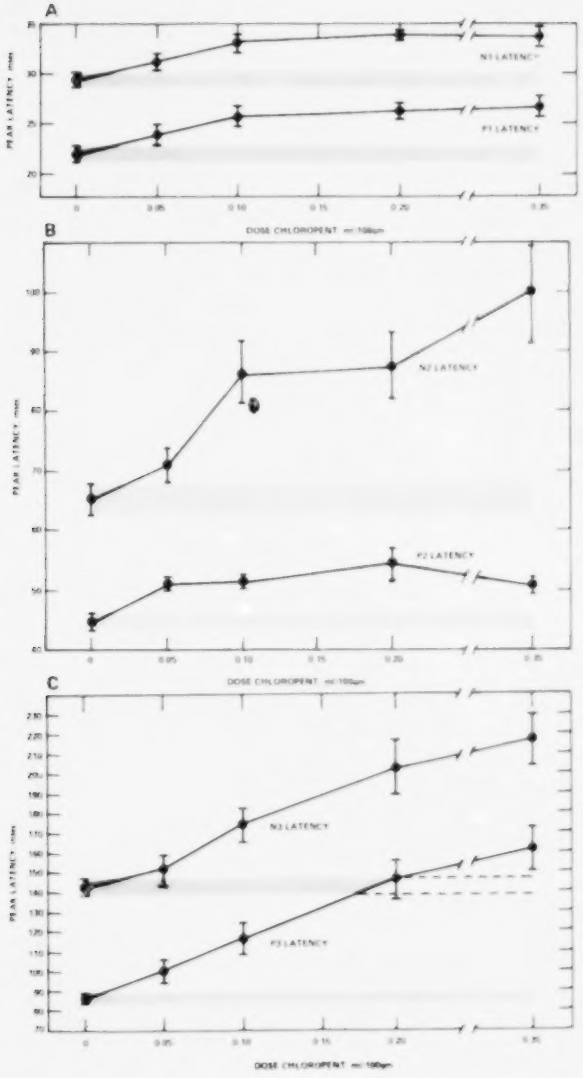

FIG. 4. Effects of chloropent anesthetic upon VER latencies. A, P1 and N1 latencies; B, P2 and N2 latencies; C, P3 and N3 latencies. Vertical bars in this and succeeding figures represent ±1 SEM.

for P3), but further increases in dosage produced further increases in latency. At the highest dosage used in this phase of the study (0.20 ml/100 g), N2, P3 and N3 latencies increased by 37%, 73% and 44% compared to control. This description is supported by the statistical evaluation of the data. The MANOVA for latency was significant, $F(18,95) = 5.30$, $p<0.0001$, and the ANOVAs for individual peaks latencies were all significant, all F's$(3,38)>5.99$; all p's<0.002.

Effects of Anesthetic Upon Amplitudes

The influence of changing levels of Chloropent upon amplitude of the different components is illustrated in Table 1, which presents peak-to-peak amplitudes as a function of anesthetic concentration. P2N2 amplitudes followed an inverted U-shaped function, increasing at low dosages and returning to control levels at 0.20 ml/100 g. P3N3 amplitude decreased in a dosage-dependent manner. The MANOVA which tested the effects of anesthetic upon amplitudes did not reach statistical significance, $F(15,98)=2.19$, $p<0.0132$, since the required p value after Bonferroni correction was 0.0125.

Effects of Hypothermia Upon Latencies

Hypothermia produced increases in the latency of all peaks which appeared linearly related to the rectal temperature. These changes are illustrated in Fig. 5. The slope of the function increased with increasing time from the stimulus, such that the N3 latency appeared much more affected (in terms of msec increase/degree) than the P1 latency. If the hypothermia-related latencies were represented as a percentage of the 37°C values, the magnitude of change was similar from one peak to the next. At 31°C the latencies of the peaks P1, N1, P2, N2, P3 and N3 had increased 41, 43, 37, 33, 36 and 37%, respectively. The MANOVA which evaluated the effects of temperature upon latencies was significant, $F(18,71)=23.27$, $p<0.0001$. Subsequent ANOVAs indicated that all peaks were significantly affected by the procedure, all F's$(3,30)>6.20$, all p's<0.0021. Duncan's test indicated that all latencies were significantly greater than control at 31°C, all but N2 latencies were greater than control at 33°C, and that only N1 and P2 latencies were greater than control at 35°C.

Effects of Hypothermia Upon Amplitude

Hypothermia produced differential affects upon amplitudes of different peaks. As may be seen in Table 2, P1N1 amplitudes increased by 99% with a 6°C decrease in rectal temperature, while P2N2 amplitudes decreased by about 30%. Other amplitudes were less affected by the procedure. These changes are supported by an overall significant MANOVA, $F(15,74)=2.64$, $p<0.0031$, and significant ANOVAs for P1N1, $F(3,30)=12.34$, $p<0.0001$, and P2N2, $F(3,30)=4.92$, $p<0.0068$.

Interactions Between Gender and Hypothermia/Anesthetic

None of the 4 MANOVAs testing gender interactions were statistically significant. Thus, within the time courses studied it must be assumed that males and females were similarly affected by the temperatures and dosages of anesthetic used.

A series of post hoc analyses were performed to determine whether a systematic difference existed between data obtained during descending and ascending rectal temperature runs of the hypothermia phase of the experiment. The analyses did not reveal any significant influence of run on evoked potential parameters, amplitude MANOVA $F(5,18)=0.55$, $p>0.73$; latency MANOVA $F(6,17)=0.83$, $p>0.56$, nor were there any run × rectal temperature interactions, amplitude MANOVA $F(15,182)=0.85$, $p>0.61$; latency MANOVA $F(18, 179)=0.72$, $p>0.79$.

DISCUSSION

The study demonstrates that, over the ranges employed, hypothermia and dosage of Chloropent have different effects upon latencies and amplitudes of flash-evoked potentials. This finding, which is probably related to different mech-

TABLE 1

EFFECTS OF CHLOROPENT UPON FLASH EVOKED POTENTIALS
MEAN (±SEM) PEAK-TO-PEAK AMPLITUDES (μV) AT NORMOTHERMIA (37–38°)

Variable	Chloropent Dosage (ml/100 g b.w.) Saline	0.05	0.10	0.20
P1N1	36.0 ± 5.9	41.3 ± 6.0	44.0 ± 6.6	52.6 ± 10.4
N1P2	93.1 ± 12.8	98.2 ± 13.9	110.7 ± 18.4	99.1 ± 15.2
P2N2	58.7 ± 7.3	95.0 ± 14.0	94.1 ± 9.0	76.0 ± 11.7
N2P3	31.7 ± 4.0	56.2 ± 12.2	41.5 ± 6.3	41.4 ± 7.4
P3N3	73.9 ± 8.5	68.1 ± 11.0	54.1 ± 6.3	57.3 ± 7.5

TABLE 2

EFFECTS OF HYPOTHERMIA UPON FLASH EVOKED POTENTIALS IN CHLOROPENT-ANESTHETIZED (0.35 ml/100 g) RATS
MEAN (±SEM) PEAK-TO-PEAK AMPLITUDES (μV)

Variable	Rectal Temperature 37°C	35°C	33°C	31°C
P1N1	44.1 ± 9.0	60.9 ± 10.3	76.1 ± 10.6	87.7 ± 11.9
N1P2	71.2 ± 10.9	78.7 ± 11.8	84.2 ± 11.9	86.7 ± 11.8
P2N2	59.5 ± 6.4	52.6 ± 4.6	49.4 ± 4.9	42.4 ± 3.2
N2P3	52.6 ± 5.8	44.1 ± 5.2	44.7 ± 5.7	38.5 ± 4.2
P3N3	45.9 ± 3.3	42.1 ± 3.6	42.8 ± 4.7	42.5 ± 3.1

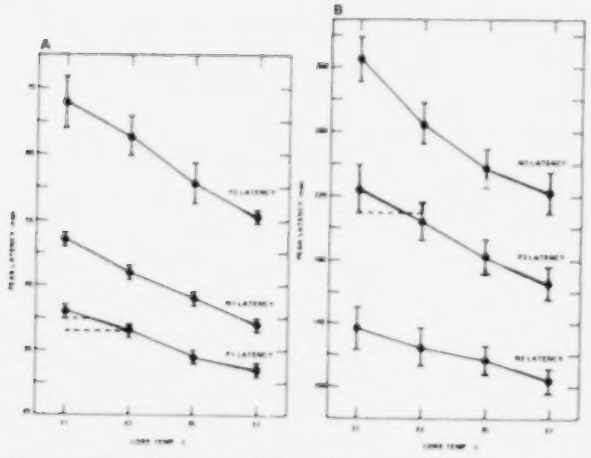

FIG. 5. Effects of hypothermia upon VER latencies. A, P1, N1 and P2 latencies; B, N2, P3, and N3 latencies.

anisms of action of hypothermia and anesthetics, has important implications for evoked potential research.

Alterations in Latencies

In the absence of selective loss of fibers, alterations in latencies of evoked potential peaks may be taken to reflect: (1) changes in conduction velocity; (2) changes in synaptic delay; or (3) some combination of these factors. In addition, observed changes may be general, applying equally to all brain areas, or regional, affecting some areas more than others.

Chloropent increased latencies of all peaks, but latencies of late peaks (N2, P3, N3) were considerably more affected than early peaks (P1, N1, P2), in terms of both absolute time (number of msec) and percentage change from control. The effect of this anesthetic on early latencies reached asymptote at low dosages, while the changes in late peaks did not appear to reach asymptote within the range of dosages used. It is unlikely that decreased conduction velocity played a role in the Chloropent-induced changes. Although high dosages of anesthetic are known to produce slight alterations in conduction velocity, mild anesthesia is generally presumed to be ineffective [20]. Therefore, the effects of anesthetic upon evoked potential latencies described here probably reflect only synaptic changes. It seems likely that regional differences exist in the synaptic sensitivity to choropent, since with increasing dosages, early peaks changed little while late peak latencies continued to increase. Regional differences in sensitivity to anesthetics have been reported previously for somatosensory evoked potentials [21]. The mechanisms by which regional differences in sensitivity appear are unknown, although they may well reflect differences in neurotransmitter systems [1,17].

The practical implication of the differential sensitivity of early and late peaks to chloropent anesthetic is clear. Studies designed to use Chloropent-anesthetized preparations for investigations of variables which may affect the primary (early) peaks of the flash-evoked potential need not be as concerned with fluctuations in level of anesthetic as studies designed to investigate late peaks. Also, if evoked potentials are used to assess level of surgical anesthesia, then later

peaks should be measured if possible. Changes in early peak latencies asymptote at anesthetic levels below critical ranges.

The data obtained during the hypothermia experiment must be interpreted cautiously since all hypothermic animals were also anesthetized. The hypothermia-induced changes in latency were by necessity added onto the anesthesia-induced changes in latency, and while the absolute latencies achieved would most certainly have been different, and the slope of the function relating latency change to temperature change may have been different in unanesthetized rats, it is unlikely that qualitatively different results would have been obtained in unanesthetized hypothermic rats. Hypothermia may deepen the level of anesthesia [18], but the findings from the anesthesia phase of this study suggest that such a result would not further affect early peak latencies. The different peaks of the flash evoked potential showed none of the regional selectivity for hypothermia that was found for anesthesia. When assessed as percentage of normothermic control values all peak latencies appeared similarly affected by hypothermia (6.3 ± 0.8%/°C).

Alterations in Amplitudes

Alterations in evoked potential amplitude may reflect (1) changes in synchrony of contributing potentials; (2) changes in size of the population of contributing neurons (e.g., loss of input fibers); (3) selective changes in populations of excitatory or inhibitory neurons; or (4) changed amplitude of individual potentials contributing to the mass recorded response.

Chloropent did not produce changes in amplitude which were statistically significant. This was surprising for two reasons: (1) previous studies have reported that other anesthetics increased the amplitude of early peaks [13,19]; and (2) the P3N3 peak is often interpreted as a reflection of arousal level [15], which most certainly changes with progressive anesthesia. Trends in the P3N3 data from this study are consistent with previous findings, but they are not supported statistically.

The present data support previous reports which have described hypothermia-induced increases in amplitude of evoked potentials [2]. In contrast to the latency data, the effect of hypothermia upon amplitudes depended upon which peaks were examined. Only P1N1 amplitudes were significantly increased by hypothermia. In this context the P1N1P2 wave behaves similarly to (1) single fiber action potentials recorded from peripheral nerves which increase in amplitude when the peripheral nerve is cooled (D. L. Jewett, personal communication), and (2) local cooling at the recording site of compound nerves [16] which increases amplitude of the compound response. The mechanism by which these changes occur is not known, but the fact that they occur in either isolated nerve fibers or bundles of axons suggests that invocation of released inhibition and/or increased excitation is not necessary to account for increased amplitude.

The observed hypothermia-induced changes almost certainly underestimate the role of temperature in VER studies, since the temperatures recorded here were rectal, not brain temperatures. Brain (and retinal) temperatures are obviously more critical variables for altering VER parameters, and while they may ultimately achieve rectal levels, more time would have been required than was allowed in this study [4].

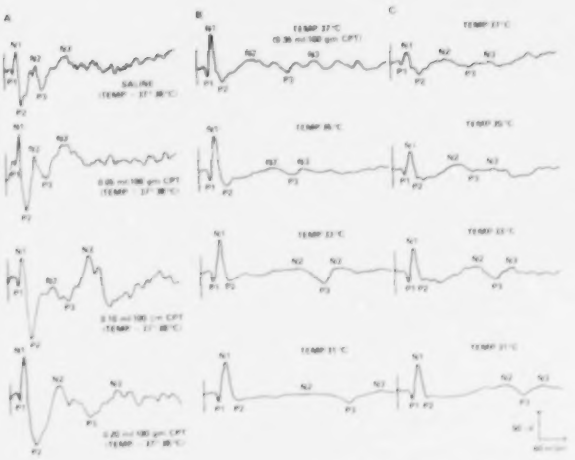

FIG. 6. Averaged waveforms recorded from a single rat during all stages of the experiment. (A) increasing concentration of Chloropent; (B) descending rectal temperature run at 0.35 ml/100 g Chloropent; (C) ascending rectal temperature run at 0.35 ml/100 g Chloropent.

Finally, it is important to acknowledge that in a study like the present one, where large distortions in waveform are the expected treatment effects, that errors in peak identification may occur. In the present study the authors were relatively confident in the accuracy of peak selection, because changes in waveform could be traced through progressively higher dosages of anesthetic for each animal. Absolute certainty in peak identification could only be achieved if independent means were available for evoking each peak. Such is not yet the case for the flash-evoked potential. Figure 6 indicates the waveforms obtained from a single animal in all phases of the study, to illustrate the manner in which peak "movement" may be traced.

In summary, this study demonstrates that Chloropent produces hypothermia, that both Chloropent and hypothermia increase VER latencies and that hypothermia, but not Chloropent, increases P1N1 amplitude. Whether other anesthetics produce the same pattern of changes as Chloropent in the flash evoked potential remains to be determined. If there is any generality between this anesthetic mixture containing sodium pentobarbital and the use of sodium pentobarbital alone, then failure of previous studies to control body temperature may have inadvertently ascribed hypothermia-related evoked potential changes to those of the anesthetic. Therefore, great care must be taken to ensure constant temperature in animals used to investigate brain function with quantitative neurophysiological techniques.

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